



Comparison of two microscopic methods for the detection of parasitic infection in antenatal women in selected areas

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ABSTRACT

Background: Soil-transmitted helminths infections are a major health problem all around the globe. According to WHO 3.5 billion people, affect and 2 million people caused morbidity (Lohe et al., 2010). In Maharashtra state, a low level of hygienic practices, bad quality of water supplies is responsible for parasitic infection. The microscopic examination of stool specimens was essential for the diagnosis of intestinal parasites (Basak, Singh, and Rajurkar, 2016). Light microscopy is typically performed in the laboratory settings far from where most STH- infected person resides, but the new diagnostic tool is invented known as foldscope (Khanam et al. 2019) and (M. Khatib et al., 2018). Foldscope is portable and easy to use in the field. **Methodology:** The study was carried out in Maharashtra, India. Total 170 antenatal women were enrolled and out of which 166 stool sample were collected. 166 stool samples were examined by iodine and saline mount under Foldscope and conventional Microscope. **Result:** Of the 166 sample, 15 sample positive for soil-transmitted helminth in conventional microscopy and 6 sample positive for foldscope microscopy. **Conclusion:** Conventional microscopy was found to be the most sensitive method for the diagnosis of soil-transmitted helminth parasites. Foldscope require improvement in magnification.

Keywords: Antenatal women, parasites, foldscope, microscopy.

1. INTRODUCTION

Soil-transmitted helminths are intestinal parasites which include roundworm (*Ascaris lumbricoides*), whipworm (*Trichiura trichuris*), hookworm (*Ancylostoma duodenale* and *Necator americanus*), *Entamoeba histolytica*, and *Giardia lamblia* (Thow et al., 2017). Some Helminths live as a parasite, or aquatic and terrestrial environment, free of the host (Puri et al., 2017). The incidence rate of soil-transmitted helminth differs from region to region, it directly depends on the personal hygiene level, quality of water supply by government, poor disposal and also depends on which method is using for the diagnostic purpose (Parameshwarappa, Chandrakanth, and Sunil, 2012) and (Agrawal, 2009). Long term STH control and elimination require improvement to water and sanitation practices. Globalization and migration have induced the spread of infection (Intra et al., 2016) and (Basak, Rajurkar, and Mallick, 2014). According to WHO, 3.5 billion people infected world wide and 450 million people cause morbidity. *Giardia intestinalis*, causing giardiasis, is the most common protozoan parasite worldwide as stated by (Shahid et al., 2014). Amoebic dysentery and liver abscess caused by *Entamoeba histolytica* resulting in 100,000 deaths annually (N. Khatib et al., 2014). *Giardia lamblia* is considered one of the non-viral causes of diarrhea in the industrial area (Verweij et al., 2004) and (Kuphal and Bosserhoff, 2009). Parasites can multiply inside the human body and spread through the water supply (Intra et al., 2016). Antenatal women, young children in developing countries show the highest incidence rate of parasitic infection. It is associated with diarrhea, dysentery, weight loss, malnutrition, anemia, abdominal pain (Khan et al., 2014) and (A and F, 2016). The history of Soil-transmitted parasitic infection in the USA and Japan where WASH advancement acted in concert with deworming to defeat STH as a public health problem. WASH intercession are differing, potentially including enhancement in water access (e.g., water purity, water quantity, and far from to water), sanitation access (e.g., access to improved latrines, latrine maintenance, and faecal sludge management), and hygiene (hand washing etc.) (Strunz et al., 2014) and (M. N. Khatib et al., 2015). Conventional microscopy is an essential tool for clinical laboratory and public health centres (K Sharma et al., 2013). This basic technology is not routinely available where soil-transmitted helminths infected person resides for diagnosis of helminths infection (Ephraim et al., 2015) and (Gondivkar et al., 2011). The accurate diagnosis of parasitic infection depending on the macroscopic and microscopic technique (Intra et al., 2016). However, a simple wet mount microscopy method lacks sensitivity if only a single sample is examined because only a few numbers of helminths eggs, unequally excreted in a single day (Khanam et al., 2013) and (Selokar et al., 2011). Sometimes it cannot be detected in the little amount of sample, it shows a negative impact on the method (Knopp et al., 2008) and (A. M. Gaidhane et al., 2008). Hand held microscopes and more recently discovered, foldscope have been used in field settings for the diagnosis of soil-transmitted helminths. Foldscope has an advantage because it is simple in use and being portable. It provides an inexpensive

diagnosis in an underserviced location (Tambekar et al., n.d.). Such modification or innovation provides low-cost laboratory instrument to underserviced location and may have used in clinical and public health centres (Ephraim et al. 2015).

The aim of this study was to evaluate the sensitivity and specificity of the foldscope and conventional microscope.

2. METHODOLOGY

Study design

The study was conducted in the selected district in Maharashtra and this study was done with full cooperation of local community. The fieldwork involved door to door visit. In this study we only focus on antenatal women, it is done by encouraging participant. Verbal consent was obtained from pregnant women. Economic background, health status, toilet facilities, water facilities, child's nutritional status, local treatments, and previous parasitic infections were collected and also name, sex, age, education, nutrition, and family relationship details were collected. Fresh stool sample was collected.

Participants

Antenatal women

Collection of samples

This study was based on epidemiological study on estimating the burden of soil-transmitted helminth infection in antenatal women in selected state of India. A total 166 stool sample was collected from household of antenatal women. The sample was collected by enrolling of antenatal women. After labelling, stool container was handover to pregnant women to collect the sample in the morning. The container of stool sample was collected during visit in the next morning. Within 3 hours, samples were transported to the Research and Development department of Datta Meghe Institute medical college Sawangi, Wardha. Each one of the samples was investigated by following methods.

Test methods

Macroscopic observation

The colour, consistency and the nature of the faeces were recorded. The stool specimens were examined for the presence of worms like Ascaris, Enterobius, adult Hookworm and Trichuris, either with the naked eye or with the aid of a hand lens.

Microscopic Examination

A little amount of sample was taken and place on a clean glass slide and add 2-3 drops of saline and iodine on separate slide then place cover slip on it, this slide was observe under conventional microscope and then the same slide was fixed by transparent nail polish to prevent leakage, it could be damage foldscope. After fixing, the slide was observed under foldscope and images were captured by Google pixel smart phone. Foldscope has magnification of 140X and it was also coupled with LED illuminator, it provides light source.

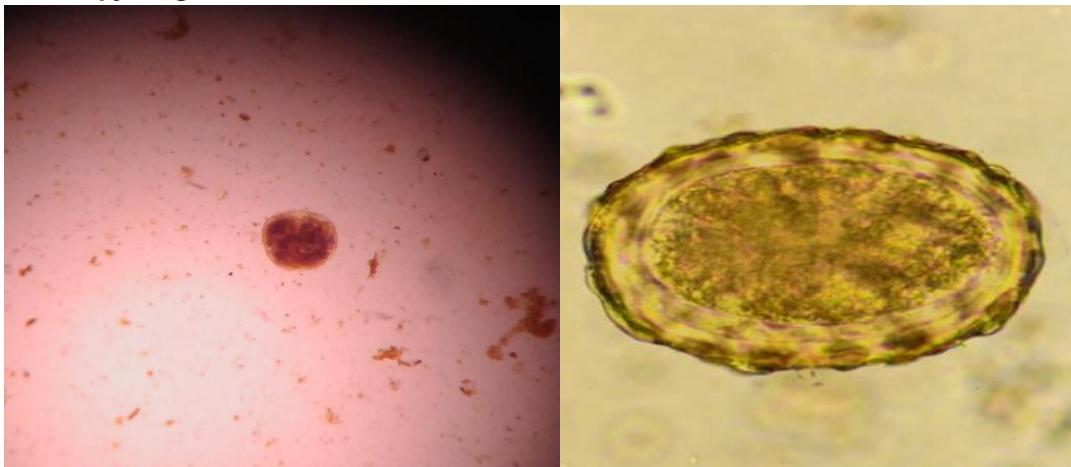
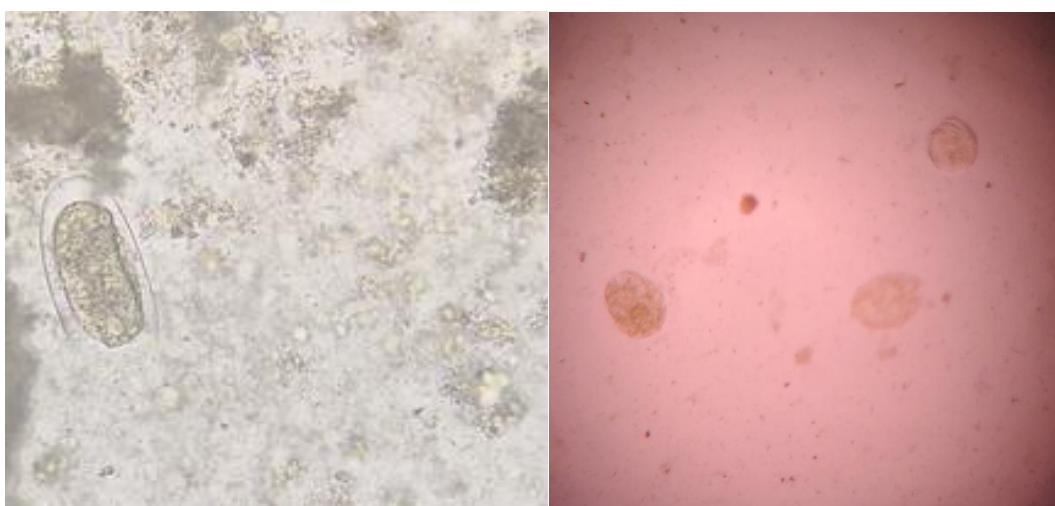
Ethical statements

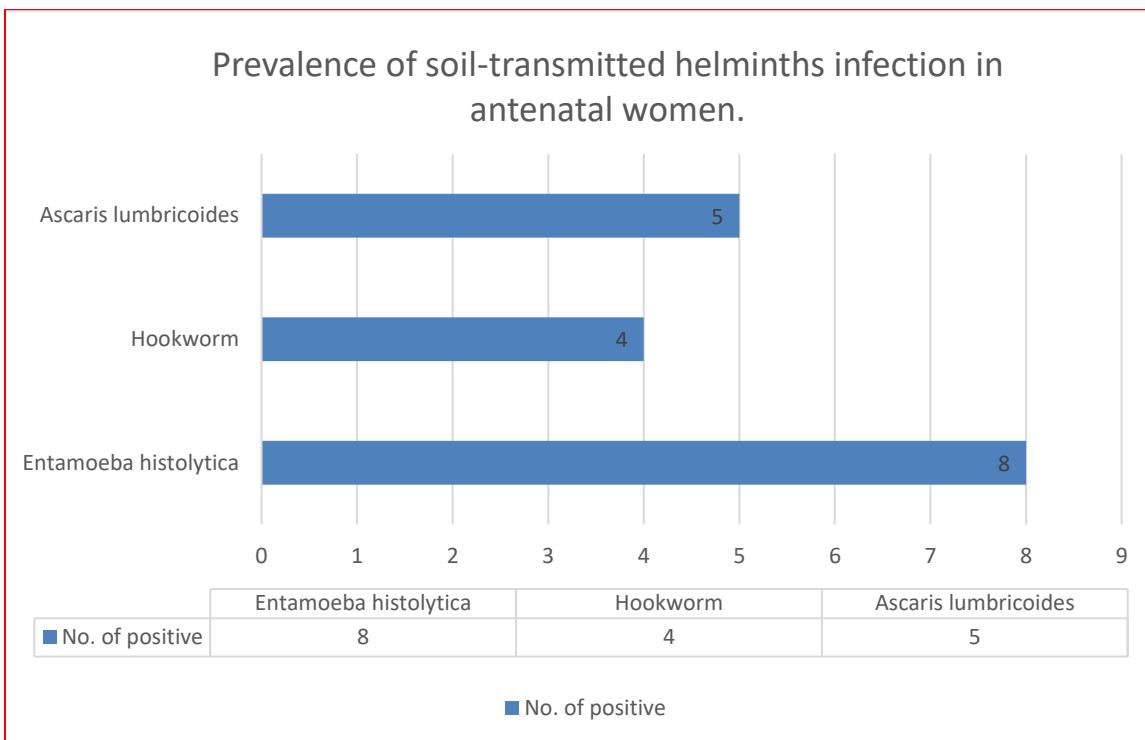
The overall study was approved by the ethics committee of Datta Meghe Institute of Medical Science, Sawangi, Wardha.

Ref. No. DMIMS(DU)/IEC/2014-2015/1202

3. RESULT

A total 166 stool sample collected from antenatal women was examined. Among these, 15 samples were positive for soil-transmitted helminths infection. The prevalence rate of *Entamoeba histolytica* was highest (53.33%) followed by *Ascaris lumbricoides* (33.33%) and followed by Hookworm eggs (13.33%). In this study, 15 parasites were detected by conventional microscopy and 6 parasites were detected by foldscope microscopy. Sensitivity of conventional microscopy shows 100% sensitivity as compare to foldscope microscopy (Figures 1 – 6, Graph 1 & Tables 1-2).

Foldscope Microscopy Images**Figure 1** Unfertilized Eggs of *Ascaris lumbricoides***Figure 2** Binucleate Cyst of *E. histolytica***Conventional Microscopy Images****Figure 3** Eggs of Hookworm**Figure 4** Fertilized eggs of *Ascaris lumbricoides***Figure 5** Eggs of Hookworm**Figure 6** Cyst of *E. histolytica*



Graph 1 Prevalence of soil-transmitted helminths infection in antenatal women.

Table 1 Foldscope microscopy as a Gold standard & conventional microscopy as a test variable

		Foldscope Microscopy			
			Positive	Negative	Total
Conventional Microscopy	Positive	06	09	15	
	Negative	00	151	151	
	Total	06	160	166	
	Sensitivity = 100% Specificity = 94.33%				
		TP- 06, FP- 09, FN- 00, TN- 151, PPV- 40%, NPV- 100%			

. roctab foldscope_microscopy conventional_microscopy,detail

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-
(>= Negat..)	100.00%	0.00%	3.61%	1.0000	
(>= Posit..)	100.00%	94.38%	94.58%	17.7778	0.0000
(> Posit..)	0.00%	100.00%	96.39%		1.0000

Obs	ROC Area	Std. Err.	—Asymptotic Normal— [95% Conf. Interval]	
			0.95397	0.98978
166	0.9719	0.0091		

Table 2 Conventional microscopy as a Gold standard & foldscope microscopy as a test variable

Conventional Microscopy				
		Positive	Negative	Total
Foldscope Microscopy	Positive	06	00	06
	Negative	09	151	160
	Total	15	151	166
Sensitivity=40%				
Specificity = 100%				
TP- 06, FP- 00, FN- 09, TN- 151, PPV- 100%, NPV- 94.37%				

. roctab conventional_microscopy foldscope_microscopy,detail

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-
(>= Negat..)	100.00%	0.00%	9.04%	1.0000	
(>= Posit..)	40.00%	100.00%	94.58%		0.6000
(> Posit..)	0.00%	100.00%	90.96%		1.0000

Obs	ROC Area	Std. Err.	—Asymptotic Normal—	
			[95% Conf. Interval]	
166	0.7000	0.0655	0.57169	0.82831

4. DISCUSSION

Soil-transmitted helminths infection is the major problem which causes morbidity and mortality in developing country like India (Shivakumar et al., 2017). The data which provide prevalence and sensitivity of various techniques help the microbiologist and public health worker for diagnosis. According to (Tambekar et al., n.d.) only saline and iodine mount has been used for the diagnostic purpose of soil-transmitted helminths no other technique has not been used, this study has done in Libya (Gondivkar et al., 2019) and (Quazi et al., 2010). Foldscope, a simple and inexpensive diagnostic tool needed for the field purpose. Foldscope microscope may have a very important role in the medical field (Gupta and Bhake, 2018) and (A. Gaidhane et al., 2008). In this study we have used two diagnostic tools one is a conventional microscope and the second is a foldscope microscope (Mittal, Jagzape, and Sachdeva, 2018). The foldscope had limited sensitivity, but this device is best for public health workers. This device uses an LED illuminator for the light source, and it magnifies directly 140X. The drawback of this device, it magnifies directly 140X and soil-transmitted helminth were found in 10X and 40X. The weight of the foldscope is smaller than 8 gm and cost only 1 \$, the sensitivity of this device may be low because of manually manipulated the lens.

Diagnosis of soil-transmitted helminths infection is very challenging and it requires a very skilled person to identify and differentiate between two parasites (Vinaya Bhat et al., 2017). Sometimes routine diagnosis lack sensitivity if the parasitic infection is less, if we want to use only a foldscope for the diagnosis then this device gives a false negative report (Balwani et al., 2019). For better results, the conventional microscope has used. In this study, the focus only on pregnant women resides in a rural area (A. M. Gaidhane et al., 2013). Entamoeba histolytica (53.33%) had a higher prevalence rate as compared to other parasites. The same result was found in 1 and 2. The most common soil-transmitted helminths infection was that of Entamoeba histolytica (53.33%) followed by Ascaris lumbricoides (33.33%) and this result was comparable to 1. Various studies have been done on school children and adult men. In this study, 15 parasites were detected by saline and iodine mount using foldscope and a conventional microscope.

5. CONCLUSION

The incidence of soil-transmitted remains high in the studied antenatal women. It is due to poor hygienic conditions, poor disposal of sewage and availability of the low quality of drinking water in the tropic and sub-tropic areas. This study shows conventional microscopy was the best diagnostic method as compare to foldscope microscopy. The sensitivity of conventional microscopy is greater than the foldscope microscopy. This study also indicated that foldscope provides alternative to conventional microscopy but foldscope require modification in magnification lens, so it can be used in field purpose for diagnosis of soil-transmitted helminths. Because it having only 140X magnification and parasite seen only on a 10X or 40X lens.

Conflicts of Interest:

The authors declare no conflict of interest.

Financial resources of the study

Department of Biotechnology, Government of India.

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